

AMENDMENTS TO THE CLAIMS

Claim 1 (currently amended): A method ~~[[of]]~~ for detecting drug resistance of HIV in a sample, comprising:

taking a culture of recombinant cells in which at least one of the recombinant cell comprises a reporter sequence comprising a promoter, an HIV-specific enhancer sequence and a reporter gene whose expression is regulated by binding of a HIV specific protein to the HIV-specific enhancer sequence ~~specific to HIV,~~

CD4, and

one or more cell surface co-receptors for HIV, wherein the one or more cell surface co-receptors are each encoded by a heterologous sequence and expressed at an elevated level relative to the level of the corresponding cell surface co-receptor naturally expressed in a human cell such that productive infection of the recombinant cell by HIV is achieved, which is defined by HIV replication and the infection of non-virally-infected cells in the culture of the recombinant cells;

contacting the cell culture with a first sample containing HIV or with a second sample containing reference HIV strain;

adding an anti-HIV agent to the cell culture for the first sample and to the cell culture for the second sample; and

determining the level of expression of the reporter gene for the first sample and for the second sample, wherein no change or an increase in the expression of the reporter gene in the first sample compared to the expression of the reporter gene in the second sample indicates drug resistance of HIV in the sample. ~~detecting a change in a level of expression of the reporter gene in the cells in the culture.~~

Claim 2 (original): The method according to claim 1, wherein the reporter gene expression is up-regulated by the HIV specific protein.

Claim 3 (original): The method according to claim 1, wherein the HIV specific protein is an HIV transactivator protein.

Claim 4 (original): The method according to claim 3, wherein the HIV transactivator protein is Tat.

Claim 5 (canceled).

Claim 6 (currently amended): The method according to claim 1 [[5]], wherein the HIV specific transactivator protein is Tat and the HIV-specific enhancer sequence comprises at least one copy of Tar sequence.

Claim 7 (original): The method according to claim 6, wherein the HIV-specific enhancer comprises at least two copies of TAR sequences.

Claim 8 (original): The method according to claim 1, wherein the reporter gene is selected from the group consisting of genes encoding β -galactosidase, luciferase, β -glucuronidase, chloramphenicol acetyl transferase (CAT), fluorescent protein, secreted embryonic alkaline phosphatase (SEAP), hormones and cytokines.

Claim 9 (original): The method according to claim 1, wherein the one or more additional cell surface receptors expressed by the recombinant cell are selected from the group consisting of CXCR4, CCR5, CCR1, CCR2B, CCR3, CCR4, CCR8, CXCR1, CXCR2, CXCR3, CX₃CR1, STRL33/BONZO and GPR15/BOB.

Claim 10 (original): The method according to claim 1, wherein the one or more additional cell surface receptors expressed by the recombinant cell comprises CXCR4.

Claim 11 (original): The method according to claim 1, wherein the one or more additional cell surface receptors expressed by the recombinant cell comprises CCR5.

Claim 12 (original): The method according to claim 1, wherein the one or more additional cell surface receptors expressed by the recombinant cell comprises CXCR4 and CCR5.

Claim 13 (original): The method according to claim 1, wherein the recombinant cell expresses a sufficient number of cell surface receptors to render the recombinant cell susceptible to infection of substantially all strains of HIV.

Claim 14 (original): The method according to claim 1, wherein the recombinant cell expresses a sufficient number of cell surface receptors to render the recombinant cell susceptible to infection of substantially all subtypes or clades of HIV.

Claim 15 (original): The method according to claim 1, wherein the recombinant cell expresses a sufficient number of cell surface receptors to render the recombinant cell susceptible to infection of clinical isolate of HIV.

Claim 16 (original): The method according to claim 1, wherein the recombinant cell is a tumor cell.

Claim 17 (original): The method according to claim 1, wherein the recombinant cell is a cell which has been immortalized by introducing a gene into the cell which renders the cell line immortalized.

Claim 18 (currently amended): The method according to claim 1, wherein the recombinant cell is ~~capable of~~ effective in achieving productive infection of a clinically isolated HIV.

Claim 19 (original): The method according to claim 1, wherein the human cell is from a stable human cell line.

Claim 20 (original): The method according to claim 19, wherein the human cell line is a human T-lymphoma cell line HUT78.

Claim 21 (original): The method according to claim 19, wherein the co-receptor for HIV is CXCR4 or CCR5 and is expressed at a level of at least 2-folds of that of the CXCR4 or CCR5 naturally expressed in a HUT78 cell.

Claim 22 (original): The method according to claim 19, wherein the co-receptor for HIV is CXCR4 or CCR5 and is expressed at a level of at least 5-folds of that of the CXCR4 or CCR5 naturally expressed in a HUT78 cell.

Claim 23 (original): The method according to claim 1, wherein the human cell is a human peripheral blood cell (PBMC).

Claim 24 (original): The method according to claim 1, wherein CD4 receptor and the one or more cell surface co-receptors for HIV are expressed by an adenoviral vector transduced into the recombinant cell.

Claim 25 (original): The method according to claim 24, wherein the adenoviral vector is replication incompetent.

Claim 26 (original): The method according to claim 24, wherein the adenoviral vector has 1-100 multiplicity of infection.

Claim 27 (original): The method according to claim 24, wherein the adenoviral vector has 10-60 multiplicity of infection.

Claim 28 (original): The method according to claim 24, wherein CD4 is expressed from the E1 region of the adenoviral vector while the one or more cell surface co-receptors for HIV are expressed from E3 or E4 region of the adenoviral vector.

Claim 29 (original): The method according to claim 24, wherein the one or more cell surface co-receptors for HIV are CCR5 and CXCR4 that are bicistronically expressed from

E1, E3 or E4 region of the adenoviral vector by a splicing mechanism or via an internal ribosome entry site.

Claim 30 (original): The method according to claim 24, wherein the native E1 promoter of the adenoviral vector is replaced by an exogenous promoter for expressing CD4 or the one or more cell surface co-receptors for HIV.

Claim 31 (original): The method according to claim 30, wherein the exogenous promoter is a CMV promoter.

Claim 32 (original): The method according to claim 1, wherein the HIV contained in the first sample is a laboratory isolate of HIV.

Claim 33 (original): The method according to claim 1, wherein the HIV contained in the first sample is a clinical isolate of HIV.

Claim 34 (original): The method according to claim 1, wherein the first sample containing HIV is a blood sample of an individual with HIV.

Claim 35 (original): The method according to claim 1, wherein the first sample containing HIV is selected from the group consisting of whole blood, blood serum, isolated peripheral blood cells, T cells, spleens and bone marrow.

Claim 36 (original): The method of claim 1, wherein the HIV contained in the first sample is HIV from a clinical isolate that has been propagated in human blood cells to increase viral titer.

Claim 37 (currently amended): The method of claim 1, wherein the anti-HIV agent is added to the cell culture before the cell culture is contacted with the first sample containing HIV or with the second sample containing the reference HIV strain.

Claim 38 (currently amended): The method according to claim 1, wherein the anti-HIV agent is selected from the group consisting of ~~consisting of~~ nucleoside RT inhibitors, nonnucleoside RT inhibitors, protease inhibitors, integrase inhibitors, viral protein antagonists, capsid lockers, antisense and ribozyme oligonucleotides against HIV mRNA or viral RNA genome, decoys of TAR sequence and RRE, soluble CD4, Gag and Env protein mutants, viral entry inhibitors and fusion inhibitors.

Claim 39 (original): The method according to claim 1, wherein the anti-HIV agent is selected from the group consisting of zidovudine, didanosine, zalcitabine, lamivudine, stavudine, abacavir, nevirapine, delavirdine, efavirenz, indinavir, ritonavir, saquinavir, nelfinavir and amprenavir.

Claim 40 (currently amended): The method according to claim 1, further comprising:

~~titering~~ titering for the number of infectious HIV particles contained in the sample before contacting the cell culture with the first sample or with the second sample.

Claim 41 (original): The method according to claim 40, further comprising:
propagating the HIV contained in the sample in human blood cells to increase viral titer before contacting the cell culture with the first sample or with the second sample.

Claim 42 (canceled).

Claim 43 (currently amended): The method according to claim 1 [[42]], wherein the reference HIV strain is HIV-1/HTLV-IIIB.

Claim 44 (currently amended): A method for detecting drug resistance of HIV in a sample, comprising:

taking a first cell culture containing CD4 and one or more cell surface co-receptors for HIV at sufficient levels such that productive infection by HIV is achieved;

contacting the first cell culture with a first sample containing HIV or with a second sample containing a reference HIV strain;

adding an anti-HIV agent to the first cell culture for the first sample and to the first culture for the second sample;

incubating the first culture in the presence of the first sample and the anti-HIV agent and incubating the first culture in the presence of second sample and the anti-HIV agent for a suitable period time;

taking a second cell culture containing a reporter sequence comprising a promoter, an HIV-specific enhancer sequence and a reporter gene whose expression is regulated by binding of a protein specific to HIV to the HIV specific enhancer sequence, CD4 and one or more cell surface co-receptors for HIV at sufficient levels such that productive infection by HIV is achieved;

transferring the supernatant of the first cell culture for the first sample after the incubation to the second cell culture and transferring the supernatant of the first culture for the second sample after incubation to the second cell culture; and

determining the level of expression of the reporter gene in the second cell culture for the first sample and in the second cell culture for the second sample, wherein no change or an increase in the expression of the reporter gene in the second cell culture for the first sample compared to the expression of the reporter gene in the second cell culture for the second sample indicates drug resistance of HIV in the sample ~~detecting a change in a level of expression of the reporter gene in the cells in the second cell culture.~~

Claim 45 (currently amended): The method according to claim 44, wherein the anti-HIV agent is selected from the group consisting of ~~consisting of~~ nucleoside RT inhibitors, nonnucleoside RT inhibitors, protease inhibitors, integrase inhibitors, viral protein antagonists, capsid lockers, antisense and ribozyme oligonucleotides against HIV mRNA or viral RNA genome, decoys of TAR sequence and RRE, soluble CD4, Gag and Env protein mutants, viral entry inhibitors and fusion inhibitors.

Claim 46 (original): The method according to claim 44, wherein the anti-HIV agent is a protease inhibitor.

Claim 47 (original): The method according to claim 44, wherein the anti-HIV agent is selected from the group consisting of indinavir, ritonavir, saquinavir, nelfinavir and amprenavir.

Claim 48 (original): The method according to claim 44, wherein the first cell culture is human peripheral blood cells.

Claim 49 (original): The method according to claim 44, wherein the first cell culture contains CD4, CXCR4 and CCR5.

Claim 50 (original): The method according to claim 44, wherein the second cell culture contains CD4, CXCR4 and CCR5.

Claim 51 (original): The method according to claim 44, wherein the reporter gene is selected from the group consisting of genes encoding β -galactosidase, luciferase, β -glucuronidase, chloramphenicol acetyl transferase (CAT), fluorescent protein, secreted embryonic alkaline phosphatase (SEAP), hormones and cytokines.

Claim 52 (original): The method according to claim 44, wherein the HIV contained in the first sample is a clinical isolate of HIV.

Claim 53 (original): The method according to claim 44, wherein the first sample containing HIV is a blood sample of an individual infected with HIV.

Claim 54 (original): The method according to claim 44, wherein the first sample containing HIV is selected from the group consisting of whole blood, blood serum, isolated peripheral blood cells, T cells, spleens and bone marrow.

Claim [[52]] 55. (currently amended and canceled).

Claim [[53]] 56 (currently amended): The method according to claim [[52]] 44, wherein the reference HIV strain is HIV-1/HTLV-III_B.

Claim [[54]] 57 (currently amended): The method according to claim 44, wherein the level of the cell surface co-receptor for HIV is higher than the corresponding cell surface co-receptor for HIV naturally expressed in a stable human cell line.

Claim [[55]] 58 (currently amended): The method according to claim [[54]] 57, wherein the human cell line is a human T-lymphoma cell line HUT78.

Claim [[56]] 59 (currently amended): The method according to claim [[55]] 58, wherein the co-receptor for HIV is CXCR4 or CCR5 and is expressed at a level of at least 2-folds of that of the CXCR4 or CCR5 naturally expressed in a HUT78 cell.

Claim [[57]] 60 (currently amended): The method according to claim [[55]] 58, wherein the co-receptor for HIV is CXCR4 or CCR5 and is expressed at a level of at least 5-folds of that of the CXCR4 or CCR5 naturally expressed in a HUT78 cell.

Claim [[58]] 61 (currently amended): The method according to claim 44, wherein CD4 receptor and the one or more cell surface co-receptors for HIV contained in the first or second culture are expressed by an adenoviral vector transduced into the cells in the culture.